Br Heart J 1982; **47**: 11–8

Assessment of heart neurons in dilated (congestive) cardiomyopathy

DALMO S AMORIM,* ECKHARDT G J OLSEN

From the Department of Histopathology, National Heart Hospital and Cardiothoracic Institute, Beaumont Street, London

SUMMARY Pathological studies in seven hearts from patients with dilated cardiomyopathy have shown that the number of neurons is significantly reduced in these compared with five hearts from normal subjects. The number of ganglion cells was counted in a strip of right atrial wall between the venae cavae and sectioned serially.

The mechanism responsible for the neuronal depopulation in this stype of cardiomyopathy could not be determined. Previous viral infection may be causally related. Three hearts of patients suffering from chronic Chagas's heart disease were also studied. Depopulation of neurons was most severe in the hearts with Chagas's disease and less severe in those with dilated cardiomyopathy, though neurons were still significantly reduced in number in the latter compared with normal controls.

Despite the lack of a specific, definite cause for the depopulation of neurons, physiological evidence of parasympathetic impairment in patients with dilated cardiomyopathy is in agreement with the pathological findings. It is suggested that on the basis of our findings neuronal depopulation in some patients with dilated cardiomyopathy may be of aetiological significance.

Dilated (congestive) cardiomyopathy is characterised by ventricular failure of no detectable cause and the possibility of a multifactorial aetiology has been entertained.[1 2] In this clinical entity physiological investigations indicate that even at an early stage of the disease parasympathetic function is impaired,[3] as in patients with chronic Chagas's heart disease.

In the latter condition pathological observations have consistently shown neuronal destruction at subepicardial level and lesions in the cardiac nerves.[4 5] In normal sized hearts of these patients, physiological investigations showed abnormal heart rate responses, interpreted as an indication of neuronal degeneration in the sinuatrial region.[6]

In view of the physiological findings in patients with dilated cardiomyopathy, seven hearts from patients in whom a firm diagnosis of this condition had been made during life have been examined and compared with five hearts obtained from subjects who had died from extracardiac causes. These findings were compared with three hearts from patients with chronic Chagas's heart disease.

*Present address: University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil.

Accepted for publication 9 December 1980

Material and methods

Seven hearts were used in this study from four men and three women whose mean age was 41·5 years ±7·1 (standard error, SE) who had died from dilated cardiomyopathy and in whom complete clinical data and detailed necropsy findings were available. The clinical diagnosis was made according to accepted criteria.[7] In addition three hearts, all male, with chronic Chagas's disease, mean age 45·6 years ±8·1 (SE) were used for comparison. Five hearts from individuals having died as a result of road traffic accidents served as controls; they were all men, mean age 44·4 years ±6·0 (SE).

A technique, previously described,[4] was employed to assess the number of ganglion cells. A rectangular block of tissue selected from the posterolateral wall of the right atrium between the venae cavae was removed. This strip was divided into four blocks, all marked to ensure correct sequential orientation. The blocks were paraffin embedded and 7 μm thick sections were cut serially. Every seventh section was stained with haematoxylin and eosin and intervening random sections were stained with Miller's elastic van Gieson stain for assessment of collagen tissue. The sections were examined and analysed under light microscopy but

Table 1 *Clinical findings in patients with dilated hypertrophy*

Case no.	*Sex*	*Age (y)*	*Duration symptoms (mth)*	*Heart failure*	*Physical findings*	*Blood pressure (mmHg)*	*Electrocardiogram*
1	M	21	9	+	Gallop rhythm	110/60	ST, LVH
2	M	43	57	+	Gallop rhythm SM (MA)	120/90	ST, LBBB
3	F	35	84	+	Split P_2 SM (MA)	110/70	SR, EAF, LAHB, LQRS
4	F	67	48	+	Gallop rhythm SM (MA)	120/90	ST, LVH
5	F	62	18	+	SM (MA)	120/80	ST, FAV, LBBB
6	M	16	3	+	SM (MA) (AA)	120/80	W
7	M	47	6	+	Gallop rhythm	120/70	ST, LVH

EAF, episodes of atrial fibrillation; FAV, first degree atrioventricular block; LAHB, left anterior hemiblock; LBBB, left bundle-branch block; LQRS, low voltage QRS complex; LVH, left ventricular hypertrophy; P_2, second pulmonary sound; SM, soft systolic murmur at MA (mitral area) or AA (aortic area); SR, sinus rhythm; ST, sinus tachycardia; W, atypical Wenckebach type I block.

Table 2 *Pathological findings in hearts with dilated hypertrophy*

Case no.	*Heart weight (g)*	*RA (mm)*	*RV (mm)*	*LA (mm)*	*LV (mm)*	*TV (cm^2)*	*PV (cm^2)*	*MV (cm^2)*	*AV (cm^2)*	*RD length (mm)*	*No. of Neurons*
1	510	2	5	2	12	140	80	120	70	32	7040
2	680	4	9	4	15	140	95	115	80	30	5084
3	430	3	3	2	13	130	72	—	68	32	4494
4	590	2	4	3	15	125	75	100	70	39	4464
5	495	2	5	3	13	125	80	95	70	45	4006
6	320	1	5	—	13	110	63	92	55	33	3585
7	570	3	6	3	16	130	82	105	75	36	3281

AV, aortic valve; LA, left atrium; LV, left ventricle; MV, mitral valve; PV, pulmonary valve; RA, right atrium; RV, right ventricle; TV tricuspid valve; RD length, strip of the right atrium removed from the posterolateral wall for ganglion cell count.

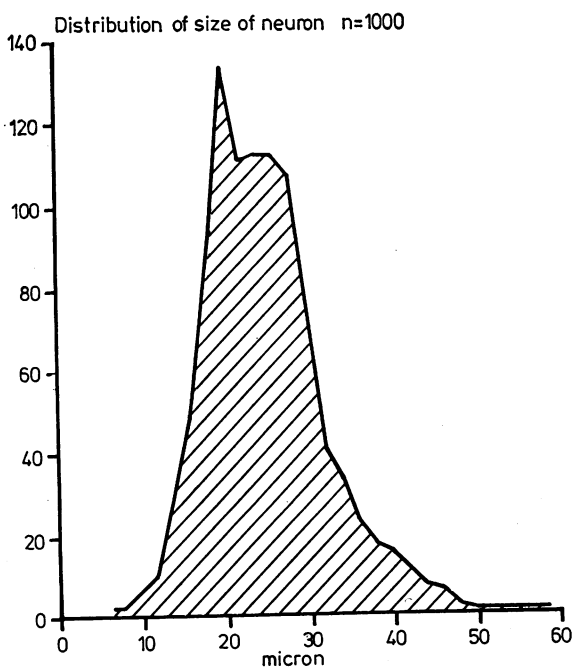


Fig. 1 *Determination of neuronal size. The size of 1000 cells, sampled from the three groups of hearts, was measured in detail by means of the Projectina microscope.*

for assessment for the size of neurons a Projectina microscope (Model 4014, Optical Works Ltd) was used. Particular attention was paid to the number of ganglion cells, to the amount of collagen tissue, and to whether or not an inflammatory infiltrate was present.

The margin of error in counting ganglion cells was assessed by determinations made by two independent observers. The statistical analysis was based on unpaired tests of significance.

Results

Clinical data and macroscopical pathological findings of patients with dilated cardiomyopathy are summarised in Tables 1 and 2.

LENGTH OF ATRIAL TISSUE

In control hearts the length of the right atrial strip averaged $35{\cdot}8 \pm 2{\cdot}7$ mm (SE) while in dilated cardiomyopathy and hearts with Chagas's disease it averaged $35{\cdot}2 \pm 1{\cdot}0$ and $29{\cdot}6 \pm 6{\cdot}6$ mm (SE), respectively.

DETERMINATION OF NEURONAL SIZE

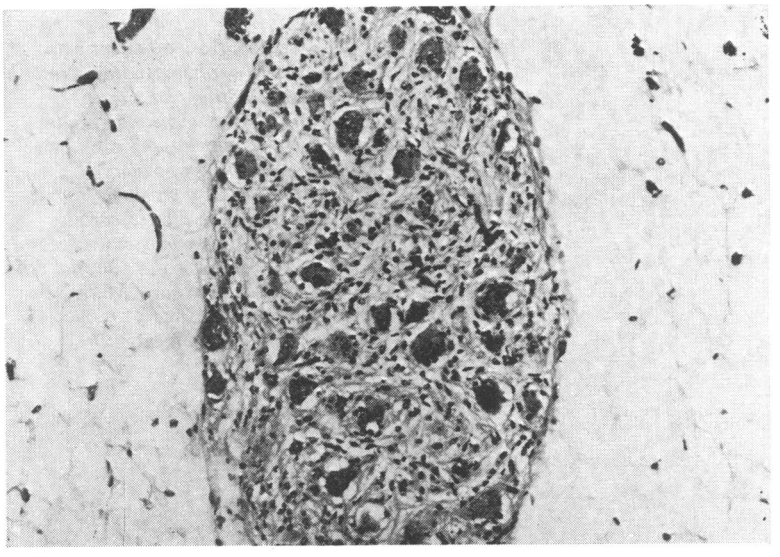
In order to ensure that neuronal cells were not counted twice, the size of 1000 cells, sampled from the three groups, was measured in detail. Results

are shown in Fig. 1. It can be seen that the larger diameter averaged $24{\cdot}8 \pm 0{\cdot}22\ \mu$ (SE). In only three of the 1000 cells did the diameter equal or exceed $50\ \mu$ (50, 52, and 58 μ), the results being obtained by direct measurement.

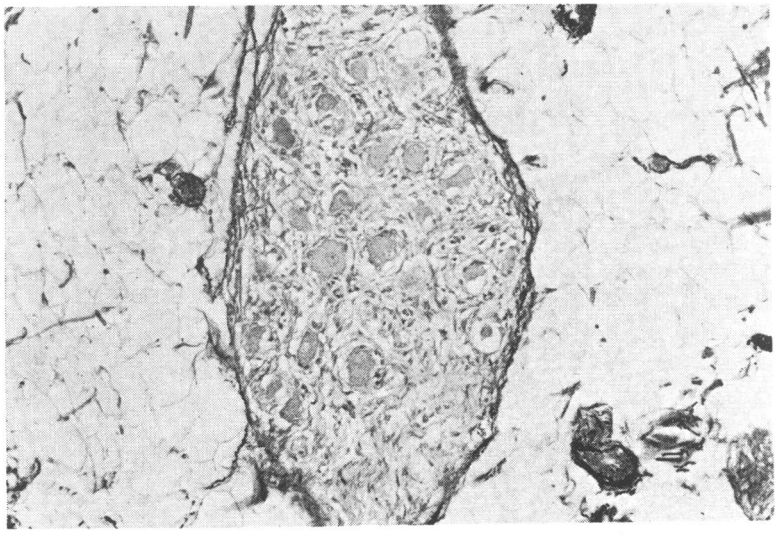
GANGLION: NUMBER OF CELLS AND STRUCTURE

A ganglion cell was considered normal if the integrity of the cell wall was intact and the cell was embedded in a delicate framework of collagen tissue, in the absence of inflammatory cells (Fig. 2A & B). In contrast, when prominent collagen tissue was present, replacing the cells to a varying degree, it was deemed abnormal (Fig. 3A & B). Inflammatory cells, as seen in hearts with Chagas's disease (Fig. 4), were not encountered in the hearts from patients with dilated cardiomyopathy.

In the control hearts the neuronal count averaged 6412 ± 377 (SE), the range being from 5180 to 7511



(A)



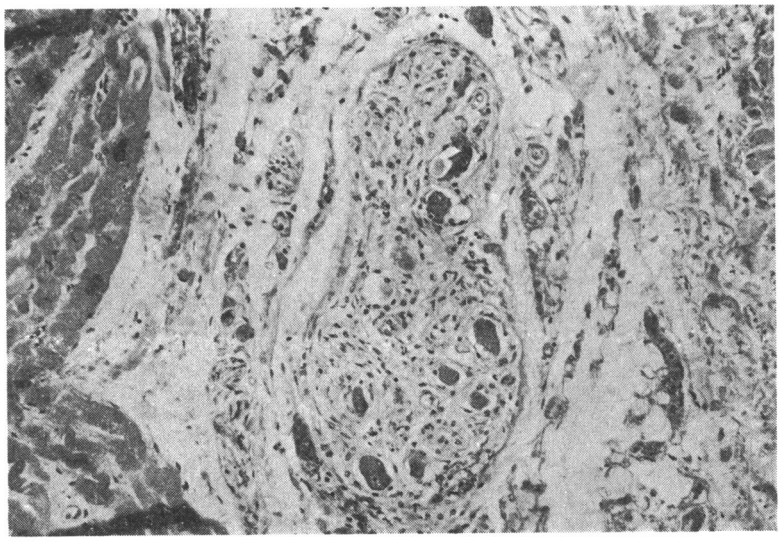
(B)

Fig. 2(A) *A normal ganglion with an evenly distributed neuronal population. (Haematoxylin and eosin. × 200 original magnification.)*
(B) *The neurons are embedded in a delicate framework of collagen tissue. (Miller's elastic van Gieson. × 200 original magnification.)*

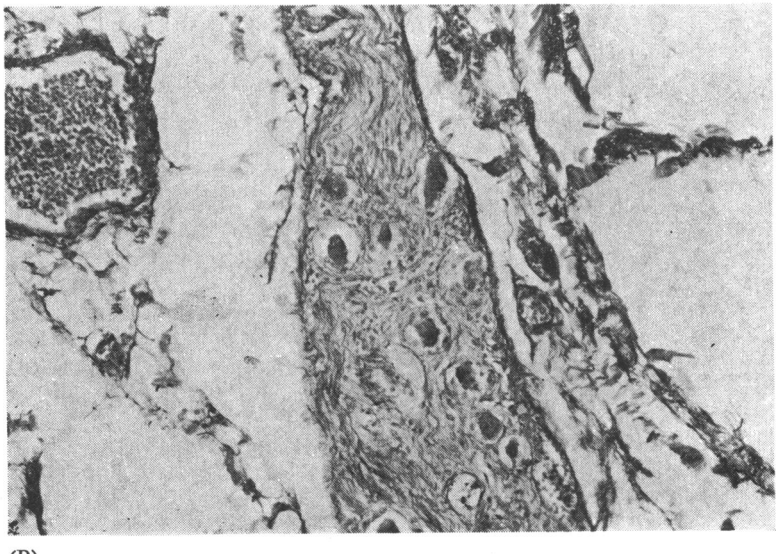
neurons. In contrast, in the hearts with dilated cardiomyopathy the number of neuron cells was significantly reduced. The average count obtained was 4565 ± 471 (SE), the range being from 3281 to 7040 neurons ($p < 0{\cdot}002$). The values for each heart are detailed in Fig. 5. It can be seen that in six out of the seven hearts the number of ganglion cells was below the lowest value obtained in the control group.

The neuronal count in hearts with Chagas's disease showed a striking reduction, with values as low as 492, 543, and 1286 in the three hearts examined.

In order to avoid a possible source of difference in the number of ganglion cells counted because of the length of the right atrial tissue examined, we calculated the number of cells per millimetre of tissue (Fig. 6). Results showed that in the control hearts an average number of $184 \pm 16{\cdot}5$ (SE) was obtained, whereas $131 \pm 17{\cdot}3$ (SE) was found in



(A)



(B)

Fig. 3(A) *Photomicrograph of a ganglion from a patient with dilated cardiomyopathy. Note the depopulation and uneven distribution of neuronal cells. (Haematoxylin and eosin. × 200 original magnification.)*
(B) *Part of a ganglion from a patient with dilated cardiomyopathy showing extensive replacement of the neuronal cells by collagen tissue. (Miller's elastic van Gieson. × 200 original magnification.)*

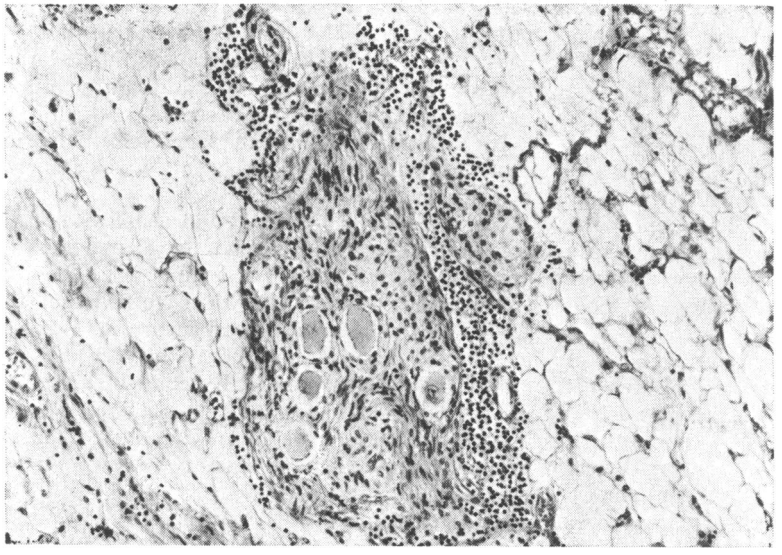


Fig. 4 *Ganglion from a patient with chronic Chagas's heart disease. Severe depopulation of neurons is present. Note also the chronic inflammatory infiltrate. (Haematoxylin and eosin. × 200 original magnification.)*

hearts with dilated cardiomyopathy ($p < 0{\cdot}05$). As can be seen in Fig. 6, in five of the seven hearts, the number of cells per millimetre of tissue was below 1 standard deviation (SD) of the mean value obtained in the controls. In hearts with dilated cardiomyopathy no correlation could be found between the neuronal counts and age, duration of symptoms, heart weight, and wall thickness. Extremely low values in the number of ganglion cells per millimetre of tissue were obtained in all the three hearts with chronic Chagas's disease (Fig. 6).

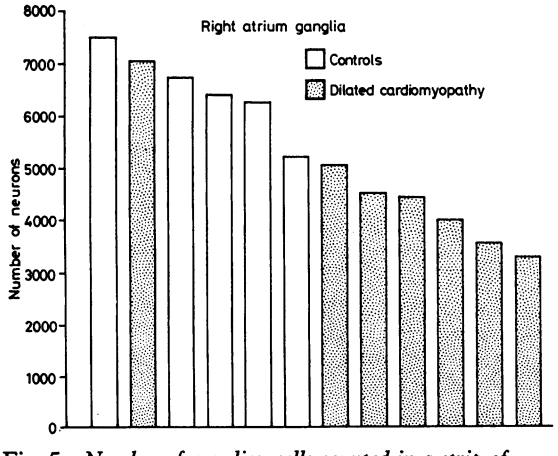


Fig. 5 *Number of ganglion cells counted in a strip of the right atrial wall between the venae cavae. In hearts with dilated cardiomyopathy the number of neuron cells was significantly reduced ($p < 0{\cdot}002$).*

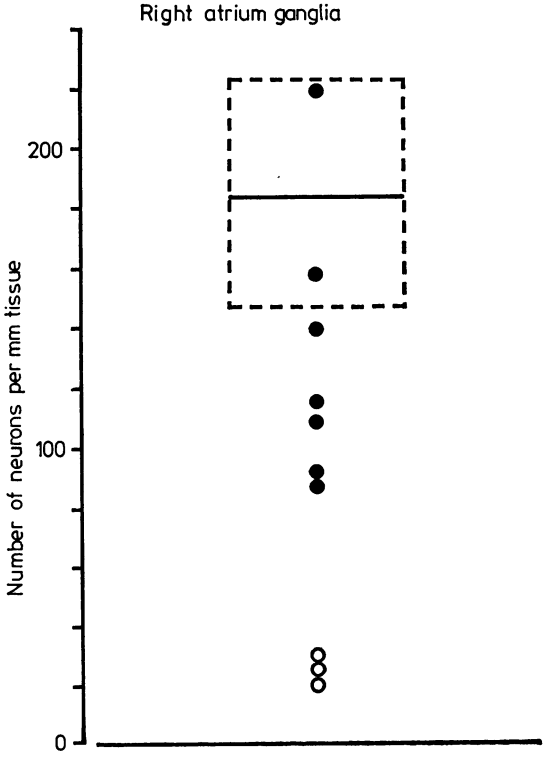


Fig. 6 *Number of ganglion cells per millimetre of right atrial tissue. The complete and dashed lines represent the average value and standard deviation of control hearts. Five hearts with dilated cardiomyopathy (solid circles) present a value less than 1 SD, while in all three hearts with Chagas's disease (clear circles) extremely low values were obtained.*

Thus, compared with the controls, the morphological abnormalities in dilated cardiomyopathy were, in particular, the amount of collagen tissue present and a severe degree of neuronal depletion.

Discussion

The diagnosis of dilated cardiomyopathy was made according to established criteria.[7] Left ventricular hypertrophy and left bundle-branch block were common (Table 1) and these, together with severe electron microscopical changes, indicate a poor prognosis.[8]

Though several factors have been suggested as being causative of or contributory to this type of cardiomyopathy the aetiology or aetiologies are so far unknown.[1]

The three patients with Chagas's heart disease presented with enlargement of the heart and clinical manifestations of heart failure lasting from six to 12 months. Impaired autonomic function has been reported in heart failure.[9 10] These changes probably occur as a direct result of a poor cardiac output and are therefore non-specific, heart failure being associated with a deficit of cardiac noradrenaline and a deficiency of sympathetic function.[9] For defective cardiac parasympathetic control no satisfactory explanation has been offered.[10] Recently we have studied patients with dilated cardiomyopathy who had a slight degree of cardiac enlargement and no recorded clinical manifestations of overt heart failure. These patients had abnormal heart rate responses during isometric exercise, impaired baroreflex sensitivity, and diminished cardiac acceleration in response to atropine-induced withdrawal of parasympathetic restraint.[3] Defective parasympathetic control has previously been reported in patients with chronic cardiac Chagas's disease who had no cardiac enlargement and no clinical or functional evidence of cardiac failure.[6]

We undertook this study in view of the similarity of the pathological and functional derangements of autonomic control in Chagas's disease and dilated cardiomyopathy.

The technique we have used ensured that no neuron was counted more than once. Our determination of neuronal size validates this basic requirement and renders any possible error negligible (Fig. 1). Furthermore, the margin of error in the number of cells was very small, because ganglion cell counts by two independent observers showed 97 per cent agreement.

Though there might be differences in cell count between different laboratories depending on the method used, these are minor and never of the order of those found between the control hearts and those with dilated cardiomyopathy. The number of ganglion cells in the cases of dilated cardiomyopathy was not reduced as severely as in the hearts with Chagas's disease but was significantly reduced in comparison with normal controls.

It could be argued that the length of atrial strips could influence the neuronal count. We have shown that this was not the case.

Macroscopically (Table 2) and histologically the accepted non-specific changes of dilated cardiomyopathy were found.[2] These differ from those in Chagas's heart disease in which thinning of the cardiac wall at the apex ("apical aneurysm"), diffuse interstitial fibrosis, and focal myocarditis are typical. The inflammatory cells frequently present in the heart ganglia in Chagas's disease (Fig. 4) were in striking contrast to hearts with dilated cardiomyopathy, in which inflammatory cells were never seen.

The mechanism of neuronal cell destruction remains undetermined. Perhaps neuronal depopulation is an accompaniment of heart failure. It could thus occur in heart disease of known aetiology such as rheumatic heart disease.

It is tempting to speculate that neuronal depletion is aetiologically related to dilated cardiomyopathy. Further studies are necessary before such a supposition can be conclusively proved.

In Chagas's heart disease degenerative lesions of the heart ganglia are well known in man as well as being found in experimental infection with *Trypanosoma cruzi*.[4 6] Comparative studies have shown an average of 4500 neurons in normal human hearts and an average of 739 (ranging from 0 to 3316) in 51 cases with chronic Chagas's heart disease.[4] Studies have also been undertaken in endomyocardial fibrosis (five cases) and in "idiopathic cardiomegaly" (nine cases),[11] a label, however, which included diverse diagnoses. Unfortunately, neither clinical data nor the duration of the illness were stated and therefore interpretation of the results is difficult. It has been suggested that in Chagas's disease neuronal destruction is produced by *T. cruzi* antigens which render lymphocytes cytotoxic to ganglion cells and that delayed hypersensitivity may be the mechanism responsible for cell destruction.[12] Sera of patients with chronic Chagas's disease contain antineuron antibodies and it has been further suggested that in chronic infections the continuous liberation of autoantigens from injured tissue may be the stimulus for the perpetuation of the autoimmune response.[13]

It is possible that in dilated cardiomyopathy a cell-mediated immune response related to an initial viral infection may exist and be causally related to neuronal depopulation. Cambridge *et al.*[14] showed

antibodies to Coxsackie B virus in some patients with dilated cardiomyopathy. Furthermore, there is evidence that in mice infected with Coxsackie B viruses an immune response develops with production of cytotoxic T cells which can damage tissues infected with virus.[15] At present the immunological implications are ill-understood and further work is necessary to establish clearly a causal relation, but in some heart diseases (for example Chagas's disease[16] and congestive cardiomyopathy[17]) there appears to be an association with immunoglobulin binding of the myocardium, and the presence of antiheart antibodies in patients' sera.

The motor nucleus of the vagus supplies preganglionic fibres which are distributed to the epicardial ganglia.[18] In man, ganglion cells in the heart are only found subepicardially in the atria, forming a plexus. From this plexus, both myelinated and non-myelinated fibres enter the heart wall, where they intermingle extensively. In our studies we have not examined the length and distribution of these fibres in the tissues of the heart, but have concentrated on the subepicardial ganglion cells.

The influence of the parasympathetic nerves on the sinuatrial node is well known, but there has been considerable controversy regarding the inotropic effect on ventricular muscle.[19] It has been shown that on stimulating the vagal nerves at constant heart rate there is only a very small negative inotropic effect on the ventricle.[20] This effect is debatable, however, and it would be interesting to explore the consequences of impairment of parasympathetic function, because the ability to alter heart rate is an important mechanism in the adjustment of cardiac output.

Our physiological findings[3] show that in dilated cardiomyopathy even at an early (prefailure) stage parasympathetic function is impaired and not associated with a significant increase in sympathetic activity. These patients, therefore, may be deprived of this sensitive mechanism for effecting rapid changes in heart rate.

Thus, we conclude that neuronal depopulation is linked with evidence of parasympathetic dysfunction in dilated cardiomyopathy. Though it has, so far, not been possible to determine the cause of neuronal degeneration in this disease, the physiological findings have a functional relevance.

D S Amorim was visiting scientist under the auspices of the Fundacao de Amparo a Pesquisa do Estado de Sao Paulo, Brazil.

The hearts with Chagas's disease were provided by Dr J S Meira de Oliveira, Ribeirao Preto, Brazil.

References

1 Goodwin JF. Congestive cardiomyopathy. Introduction, problems and aims of the multicentre research project. *Postgrad Med J* 1978; **54:** 431–2.
2 Olsen EGJ. Pathology of primary cardiomyopathies. *Postgrad Med J* 1972; **48:** 732–7.
3 Amorim DS, Dargie H, Heer K, *et al.* Is there autonomic impairment in congestive (dilated) cardiomyopathy? Lancet 1981; i: 525–57.
4 Köberle F. Enteromegaly and cardiomegaly in Chagas' disease. *Gut* 1963; **4:** 399–405.
5 Mott KE, Hagstrom JWC. The pathologic lesions of the cardiac autonomic nervous system in chronic Chagas' myocarditis. *Circulation* 1965; **31:** 273–86.
6 Amorim DS. Chagas' disease. In: Yu PN, Goodwin JF, eds. *Progress in cardiology, 1979.* Philadelphia: Lea & Febiger, 1979: **8:** 235–79.
7 Oakley C. Diagnosis and natural history of congested (dilated) cardiomyopathies. *Postgrad Med J* 1978; **54:** 440–7.
8 Kuhn H, Breithardt G, Knieriem H-J, Köhler E, Lösse B, Seipel L. Prognosis and possible presymptomatic manifestations of congestive cardiomyopathy (COCM). *Postgrad Med J* 1978; **54:** 451–9.
9 Chidsey CA, Braunwald E, Morrow AG. Catecholamine excretion and cardiac stores of norephinephrine in congestive heart failure. *Am J Med* 1965; **39:** 442–51.
10 Eckberg DL, Drabinsky M, Braunwald E. Defective cardiac parasympathetic control in patients with heart disease. *N Engl Med J* 1971; **285:** 877–83.
11 Böhm GM. Quantitative study of the intrinsic innervation of the heart in endomyocardial fibrosis and African cardiopathies. *Rev Inst Med Trop Sao Paulo* 1968; **10:** 84–7.
12 Teixeira ARL, Teixeira L, Santos-Buch CA. The immunology of experimental Chagas' disease. IV. Production of lesions in rabbits similar to those of chronic Chagas' disease in man. *Am J Pathol* 1975; **80:** 163–80.
13 Ribeiro dos Santos R, Ramos de Oliveira JC, Rossi MA. Antibodies to neurons in chronic Chagas' disease (letter). *Trans R Soc Trop Med Hyg* 1976; **70:** 167.
14 Cambridge G, MacArthur CGC, Waterson AP, Goodwin JF, Oakley, CM. Antibodies to Coxsackie B viruses in congestive cardiomyopathy. *Br Heart J* 1979; **41:** 692–6.
15 Wong CY, Woodruff JJ, Woodruff JF. Generation of cytotoxic T-lymphocytes during Coxsackie virus B-3 infection. II. Characterization of effector cells and demonstration of cytotoxicity against viral-infected myofibers. *J Immunol* 1977; **118:** 1165–9.
16 Trezza E, Tucci PJE, Buffollo E, Montenegro MRG. Anticorpos antimiocardio na doenca de Chagas crônica. Correlação entre a sorologia e a eletrocardiografia. *Arq Bras Cardiol* 1975; **28:** 327–44.
17 Bolte HD, Schultheiss P. Immunological results in myocardial diseases. *Postgrad Med J* 1978; **54:** 500–3.
18 Hirsch EF, Borghard-Erdle AM. The innervation of

the human heart. *Arch Pathol* 1961; **71**: 384–407.
19 Linden RJ. Function of nerves of the heart. *Cardiovasc Res* 1972; **6**: 605–26.
20 Furnival CM, Linden RJ, Snow HM. The response of the left ventricle to stimulation of the vagus nerves. *J Physiol (Lond)* 1968; **198**: 100–1 P.

Requests for reprints to Dr E G J Olsen, Department of Histopathology, National Heart Hospital, Westmoreland Street, London W1M 8BA.